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MEDICAL WAR MANUAL No. 6

Authorized by the Secretary of War
and under the Supervision of the Surgeon-General
and the Council of National Defense

Laboratory Methods

OF THE

UNITED STATES ARMY

COMPILED BY THE

DIVISION OF INFECTIOUS DISEASES
AND LABORATORIES

OFFICE OF THE SURGEON-GENERAL, WAR DEPARTMENT
WASHINGTON, D. C.

SECOND EDITION, THOROUGHLY REVISED

Illustrated



LEA & FEBIGER
PHILADELPHIA AND NEW YORK
1919

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Herbert A. Huntington
Third Army Laboratories

FOREWORD.

Coblenz
Germany

THIS edition of the Manual of Laboratory Methods represents a complete revision of the first edition. The chapter on special bacteriological methods has been almost entirely revised, with the addition of certain methods which have proved practicable in the Army laboratories. The chapter on sanitary examination of water and sewage has been largely revised. A complete new section on autopsy technique, with directions for preparation and shipment of museum specimens, has been added. Many minor sections have been introduced, taking advantage of the experience gained in the laboratories both in this country and in France. Where a method adopted as a standard by the Central Laboratory of the A. E. F. has differed from that used in the Army in this country, both methods are given. Special acknowledgment is made to Dr. Donald D. Van Slyke, Dr. William G. MacCallum, Captain Oswald T. Avery and Major Edward T. Tucker, for aid in the preparation of some of the larger sections.

THE EDITOR.

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The Editor.

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THE RESPONSIBILITIES OF THE LABORATORY.

The functions of the laboratory consist chiefly in helping to safeguard the health of the troops by making rapid and accurate diagnosis of infectious and other diseases, by which the division surgeon and his staff may be guided in prophylaxis and treatment.

The laboratory director should bear in mind that military laboratories must combine the functions of Health Department laboratories with those of diagnostic hospital laboratories.

The directors of such laboratories are responsible directly to the commanding officer of the camp base hospital, through whom he will coöperate with the sanitary inspector of the camp in any sanitary examinations in which the laboratory can facilitate control of camp conditions.

The director of the laboratory is responsible not only for the proper conduct of the laboratory and the accuracy of the reports which are issued, but also for the preparation of requisitions for the supplies which are used in the laboratory.

Such requisitions are made up in accordance with the *Manual of the Medical Department*, paragraph 477 to 490, and he should note especially paragraph 485.

GENERAL RULES FOR THE CONDUCT OF THE LABORATORY.

1. Wear apron or other protective covering over uniform when in the laboratory. This avoids carrying infectious material to mess.
2. Keep your desk and floor area clean.
3. Wash and disinfect your hands before leaving the laboratory.

4. Preserve all cultures, except liquefied gelatin plates, for one week before discarding.

5. Place all discarded cultures and slides in receptacles provided for that purpose. If they are not provided, obtain them.

6. Label everything and do it legibly.

7. If a culture be dropped on the floor, breaking the glass, do not clean it up until everything has been disinfected for one hour; wet a towel in antiseptic solution and cover the entire infected area.

8. Clean the oil-immersion lens before leaving the laboratory.

9. Keep all room temperature cultures in dark, dust-free closets.

10. Do not keep gas burners lighted when not in use. Do not allow sterilizers and water baths to boil dry. Do not leave stoppers out of bottles. Do not take more media, stains, and other supplies than you need.

11. Clean oil from the condenser, lens, and mirror of the dark-field apparatus before leaving for the day. If you do not, it will have to be done in the morning before the apparatus can be used, and will then be much more difficult.

12. Remember that it is as important to keep accurate records as to carry on accurate tests.

The following tabulation is suggested as a general plan for the organization of the laboratory:

1. The Chief of the Laboratory should organize his department so that he will be relieved of routine work, will have time to act as consultant on the laboratory findings to the hospital and to the Camp Surgeon, as well as have time for administrative detail.

2. It is suggested that the laboratory personnel be divided into groups with an individual with special training along a specific line in charge of each group. For types of work requiring technical skill and experience, such as pneumo-

coccus grouping, it is considered advisable to have one person responsible and held to this, so as to obtain uniformity of results.

3. The following plan for division of work is suggested: The personnel should be divided as far as possible according to training and the amount of work to be accomplished in the different groups.

(a) Pathology:

Autopsy.

Section cutting (special technician).

Preservation and shipment of museum specimens.

(b) Clinical microscopy:

Routine examination of blood, sputum, urine, feces, spinal fluid (cell counts), urethral discharges, dark field examinations, etc.

(c) Bacteriology and serology:

Preparation of media.

Bacteriology of sputum, pneumonia and streptococcus typing (special technician).

Nasopharyngeal cultures and detection of carriers of meningococcus and diphtheria.

Bacteriology of feces and urine for detection of typhoid and dysentery groups.

Bacteriology of food, milk, water and sewage.

Blood cultures.

Wassermann tests.

(d) Chemistry:

Clinical chemistry, special examination of blood, urine, etc.

Chemical examination of food, milk, water and sewage.

(e) Records and reports:

Files and librarian.

Journals and books.

(f) Photographer:

For cases, specimens, gross and microscopic.

COLLECTION AND SHIPMENT OF SPECIMENS AND MATERIALS.

SPUTUM.

THE sputum from the lower respiratory passages, and not saliva or the nasopharyngeal secretions, should be submitted. No disinfectant should be added to the specimen. It is necessary to explain this to the ward attendants, as they are usually instructed to add disinfectants to all discharges. Gross contamination should be avoided by keeping the specimen bottle tightly corked except during actual collection of the specimen. Plainly label the bottle with the patient's name, rank, organization, the station from which sent, and the examination desired. Each specimen must be accompanied by requests, *in duplicate*, on Form 550, M. D.

FECES.

The specimen bottles mentioned in this circular are for forwarding specimens of feces for examination for parasites and their ova and occult blood. If bacteriological examinations for typhoid fever, paratyphoid fever, or bacillary dysentery are desired, specimens should be forwarded in glass-stoppered bottles. Patients in whose cases an examination for occult blood is desired should be placed on a meat-free diet for at least two days prior to the collection of the specimen.

The specimens or feces are to be collected in large numbers for carrier examinations for typhoid, paratyphoid, etc. Time can be economized and a system for laboratory collections established by furnishing test-tubes containing

sterile swab sticks. The specimen feces can be taken up with a swab stick and inserted into the test-tubes, upon which are labels to be used for the name, rank and organization of the subject.

URINE.

Specimens of urine for bacteriological examination or animal inoculation must be collected under aseptic conditions. Catheterization should be resorted to and no disinfectant should be added. Specimens to be examined for organisms of the typhoid-paratyphoid group should be forwarded in bile medium. Each specimen of urine (except for typhoid) must be accompanied by requests, *in duplicate*, on Form 55m, M. D.

USE OF DIPHTHERIA CULTURE TUBES.

Good illumination of the throat is essential. Remove the swab from its container, and while depressing the tongue, pass swab into pharynx, avoiding contact with tongue or other parts of mouth, and rub firmly, but gently, over any visible membrane, or if no membrane is present, over the tonsils and pharynx. Withdraw swab and rub it over the whole surface of the Loeffler serum medium, being careful that the portion of the swab previously in contact with the membrane comes in contact with the medium. The inoculation of the medium should be thorough, but the surface of the medium must not be broken.

Replug the culture tube, return the swab to its container, pack securely in cotton, and mail to the officer in charge of the laboratory.

The culture tube must be plainly labelled with the name, rank and organization of the patient and the station from which the culture is sent. The nature of the examination desired—"for diphtheria"—must also be shown. Requests, *in duplicate*, on Form 55u must accompany each culture.

**COLLECTING AND SHIPPING SAMPLES OF MILK FOR
CHEMICAL AND BACTERIOLOGICAL EXAMINATION.**

COLLECTION OF SAMPLES.—The surgeon should request, through the commanding officer, that the delivery wagon of each dairyman supplying the command be directed to report at the surgeon's office at a designated day each month for the purpose of delivering milk samples.

A one-quart bottle should be selected at random for analysis, the bottle being labelled with the name of the dairy.

PREPARATION FOR SHIPMENT.—If samples are delivered during the early morning hours they should be placed on ice immediately. All samples should be forwarded to the laboratory on the day of collection.

The following procedure should be carried out in preparing the samples for shipment: The quart of milk must be poured 25 times between the original container and a sterile bottle or flask in order that the milk and cream may be thoroughly mixed and that clumps of bacteria may be broken up. After thorough mixing add 1 c.c. of commercial formalin to the quart (1000 c.c.) of milk and agitate thoroughly to ensure inhibition of further growth of bacteria. Then fill the sterile 60 c.c. sample bottle. When it is desired to learn the type of organisms present the formalin should be omitted.

It is essential that the bottle containing the sample be filled flush to the lower end of the stopper to prevent churning of sample with formation of butter while in transit to the laboratory. Seal with paraffin or wax and cover with a square of muslin held in place by copper wire.

Label each bottle with the name of station or command, location and name of dairy, date of collection and date of shipment. Pack securely in absorbent cotton to avoid breakage.

Samples of milk for examination must reach the laboratory prior to the 25th of each month. Milk samples other than routine may be sent to the laboratory when occasion demands.

labelled with the patient's name, rank, organization, and the station from which the specimen is sent. The test desired (Wassermann, gonococcus-fixation, etc.) should be designated also. The capsules should be wrapped securely in cotton to avoid breakage in the mails.

(g) Specimens for the Wassermann test must be accompanied by requests, *in duplicate*, on Form 55q, M.D., and the *first time* the serum of an individual is tested at *this* laboratory it must also be accompanied by a Wassermann card, Form 97, M. D.

AGGLUTINATION TESTS.—The institution of prophylactic inoculation against typhoid and paratyphoid fevers has very largely obviated the usefulness of the agglutination test (Widal) as a diagnostic procedure in these diseases. The results are of value in establishing a positive diagnosis in inoculated individuals only when there is a definite increase in the agglutinating property of the serum, as shown by repetition of the test with sera collected at intervals of a week or ten days. *For early diagnosis the blood culture should be resorted to in all cases.* In bacillary dysentery the agglutination test may be of value in establishing a diagnosis; but, as a rule, the serum possesses agglutinating properties only in the severe or moderately severe cases. Isolation of the causative organism by bacteriological examination of the feces is a surer and altogether more satisfactory diagnostic procedure.

Blood specimens forwarded for agglutination tests for typhoid and paratyphoid fevers should be collected in Wright's capsules, under aseptic precautions, in order that cultures may be attempted from the clot.

COLLECTING AND FORWARDING SPECIMENS FOR THE DIAGNOSIS OF GLANDERS.

CULTURES.—The glanders bacillus can be obtained easily, in pure cultures, from the interior of suppurating glands and

nodules, which *have not yet opened to the surface*. The discharges from the nostrils, or from an open lesion, are much less satisfactory, as very few bacilli may be present, and the detection of these is difficult because of the invariable admixture of numerous other microorganisms. Glycerin-agar slants should be used. The procedure of making cultures follows:

(a) Select a fluctuating gland or nodule which has not yet opened to the surface, shave the overlying skin, and sterilize this area with a thick coating of tincture of iodine.

(b) Incise the nodule with a sterile scalpel.

(c) Evert the edges of the incision with thumb and middle finger, introduce a sterile swab into the center of the lesion, and, exercising care that it does not touch the skin edge of the wound, rotate it gently so as to thoroughly impregnate it with the contents of the lesion.

(d) Remove the cotton plug from the culture tube, flame the neck of the tube and smear the material on the swab over the surface of the culture medium.

(e) Carefully replace the cotton plug in the culture tube, return the swab to the tube in which it was received and forward both tubes to the laboratory.

(f) The presence of *B. mallei* in material from open lesions, nasal discharge and sputum (pulmonary lesions in man) can be determined by guinea-pig inoculation (Straus reaction). Collect discharge and forward in sealed container; if necessary, add a small amount of saline to prevent drying.

(g) The tubes or containers should be plainly labelled with name (human case) or name or identification mark of animal, the nature of the material, the examination requested, together with the name, rank and station of the veterinarian to whom the reports of the examination are to be forwarded. Requests, *in duplicate*, on Form 55u, M. D., must accompany each culture.

COMPLEMENT-FIXATION OR AGGLUTINATION TEST.—A posi-

tive complement-fixation test may be obtained from the seventh to the tenth day and usually persists during the course of the disease. The agglutination test may be positive in four to seven days, the content in agglutinins increasing early in the disease, but decreasing if the disease becomes chronic. All blood-serum tests are influenced by the injection of mallein or vaccine, and blood should be taken before their administration.

▶ Blood for these tests is collected from man as per instructions under Wassermann, page 16; from horse as follows:

(a) Sterilize a large-sized hypodermic needle *by boiling*. Do not use phenol or other antiseptics for this purpose.

(b) Shave and sterilize with tincture of iodine the skin over the jugular vein.

(c) Make the vein prominent by pressing with the thumb below the area selected for puncture, thrust needle into vein and permit blood to flow into sterile bottle furnished for this purpose. Tightly cork after collection.

Stand toward the side of the horse, while drawing blood, to avoid danger from sudden rearing.

Label specimen plainly with name (human case) or name or identification mark of animal, the nature of the specimen, the test desired and the name, rank and station of the surgeon or veterinarian to whom report is to be returned. Pack the bottle securely in cotton to avoid breakage. Requests *in duplicate*, on Form 55u, M. D., must accompany each specimen.

WATER ANALYSIS, BACTERIOLOGICAL. DIRECTIONS FOR COLLECTING AND SHIPPING.

These directions are a transcription of the instructions in the Manual of the Medical Department, 1916:

356. At the time of forwarding the water the officer to whom it is sent should be advised of the following particulars: (1) The date, place and mode of shipment; (2) the date

and place of the collection of the water; (3) the character of the watershed, its topography, and the uses to which the country is put, if inhabited; (4) the proximity of houses, barns, privies, or other possible sources of contamination to the place of collection or the source of supply; (5) the proximity of fertilized land to such place or source and whether the said land is higher or lower than the adjacent land; (6) such other information as may suggest a possible deleterious influence on the purity of the water. If the water is from a well the letter should report the depth of the well, the strata found in digging or boring it, and the depth of the water in the well.

357. The specimen should, when practicable, be collected by a medical officer. If the water to be examined is delivered through pipes or is pumped from a well or cistern the local supply pipe and all pump connections should be emptied by allowing the water to run for fifteen minutes before taking the samples.

358. BACTERIOLOGICAL EXAMINATIONS.—Samples of water for bacteriological examination should be collected in bottles furnished for the purpose. Each bottle is sterilized before leaving the laboratory, and the glass stopper is protected by a piece of heavy sterilized muslin securely wired to the neck of the bottle. The stopper should not be removed until immediately before the bottle is filled.

(a) In taking specimens from a faucet or pump (after emptying the supply pipes and connections conformably to paragraph 357) a small, gentle stream should be allowed to flow, the stopper taken out, the bottle grasped near the bottom, held in an upright position, and the stream permitted to flow into the bottle until it is filled to the shoulder. The stopper should then be replaced; both it and the cloth should be secured by carrying the wire several times around the neck of the bottle and twisting the ends tight. The stopper must be handled only by the square cloth-covered top. The lip

of the bottle must not be brought in contact with the faucet or spout, nor should the neck of the bottle or naked part of the stopper be permitted to come in contact with any object during the manipulation. The projecting flange is designed to protect the plug of the stopper, which it will do if the stopper, after withdrawal, is held by the top in a vertical position. The stopper should not be laid down and the cloth should not be handled by the fingers except in the act of securing the wire about it. When well water is to be examined the bottle should be filled directly from the bucket constantly in use for drawing the water, and from no other vessel.

(b) On account of the labor involved and the possibility of error, bacteriological examinations of water collected in any other than the prescribed receptacles will not be made.

(c) Each package should be plainly marked to show the source from which the samples are taken and the date of collection.

(d) The case should be marked "Water for Bacteriological Examination," and it should be forwarded by mail at the earliest moment.

(See paragraph 355a: All bottles containing fluid material sent through the mails must be securely packed in cotton in double containers.)

PREPARATION AND SHIPMENT OF AUTOPSY SPECIMENS FOR THE MUSEUM.

See page 109

SOLUTIONS AND STAINS.

PHYSIOLOGICAL SALT SOLUTION.

For bacteriological work, physiological solution is usually made up by adding 8.5 grams of sodium chloride to a liter of distilled water. When for reasons of speed and convenience tap water is used, one should have some idea of the salt contents of the water used before relying upon it.

SODIUM CITRATE SOLUTION.

For bacteriological purposes this contains 1 per cent. of sodium citrate and 0.85 per cent. of sodium chloride.

If sodium citrate is to be used for prevention of coagulation of blood without considerably changing the volume of the blood the solution is made up to contain 10 per cent. of sodium citrate and 0.85 sodium chloride.

FIVE PER CENT. SULPHURIC ACID FOR DECOLORIZING (AS USED IN TUBERCLE BACILLUS STAINING).

Slowly allow 2.7 c.c. of c. p. sulphuric acid of a specific gravity of 1.84 to flow into 80 c.c. of distilled water. After cooling, bring volume up to 100 c.c. (St. Luke's Manual.)

ACID ALCOHOL (ORTH).

HCl	1.0 c.c.
70 per cent. alcohol	99.0 c.c.
	(23)

OXALATE SOLUTION FOR BLOOD CULTURE.

Ammonium oxalate	2.0 grams
Sodium chloride	6.0 grams
In distilled water	1000.0 c.c.

ZENKER'S FLUID.

Potassium dichromate	2.5 grams
Mercury bichloride	5.0 grams
Water	100.0 c.c.

Glacial acetic acid 5 per cent. is added to this stock solution just before use.

COPPER SULPHATE SOLUTION FOR CAPSULE STAIN.

20 grams of copper sulphate crystals are dissolved in 100 c.c. of water.

STOCK STAINING SOLUTIONS.

It is convenient in stationary laboratories to keep stock stains in the form of saturated solutions. The strengths of various saturated solutions are as follows:

	Saturation strengths.
Fuchsin in alcohol	3.0 per cent.
Gentian violet in water	1.5 "
Gentian violet in alcohol	4.8 "
Methylene blue in water	6.7 "
Methylene blue in alcohol	7.0 "
Safranin	4.0 "

Saturated alcoholic solutions can be kept and aqueous staining solutions can best be made by adding 5 per cent. of the filtered alcoholic solutions to water.

LOEFFLER'S ALKALINE METHYLENE BLUE.

Saturated alcoholic solution of methylene blue	30.0 c.c.
1 to 10,000 solution potassium hydroxide in water	100.0 c.c.

ZIEHL CARBOL-FUCHSIN SOLUTION.

Fuchsin (basic)	1.0 gram
Alcohol (absolute)	10.0 c.c.
5 per cent. phenol	100.0 c.c.

To make up this staining solution by another method, 90 c.c. of a 5 per cent. aqueous solution of phenol is mixed with 10 c.c. of saturated alcoholic basic fuchsin.

CAPSULE STAINS.—*Hiss's Copper Sulphate Method.*—Cover-slip preparations are made by smearing the organisms in a drop of animal serum, preferably beef-blood serum.

Dry in air and fix by heat.

Stain for a few seconds with—

Saturated alcoholic solution of fuchsin or gentian violet, 5 c.c., in distilled water, 95 c.c. This combination is often too weak for good results. A gentian-violet or fuchsin solution twice as strong is advantageous. Gram's gentian violet or carbol-fuchsin can be used.

The cover-slip is flooded with the dye and the preparation held for a second over a free flame until it steams.

Wash off dye with 20 per cent. aqueous copper sulphate solution.

Blot (do not wash).

Dry and mount.

NEISSER STAIN FOR POLAR BODIES.

Methylene blue	1.0 gram
Absolute alcohol	200.0 c.c.
Glacial acetic acid	50.0 c.c.
Distilled water	1000.0 c.c.

Preparations fixed by heat are immersed in this stain for five seconds and then washed in water and counterstained with Bismarck brown or, better, safranin.

CARBOL-THIONIN.

Saturated solution of thionin in 50 per cent. alcohol 10.0 c.c.
 Two per cent. phenol 100.0 c.c.
 Stain for two minutes.

GRAM'S METHOD AND MODIFICATIONS.—Preparations are made on cover-slips or slides in the usual way.

It is always necessary to control Gram stains with organisms of known type.

The preparation is covered with an anilin gentian-violet solution, which is best made up freshly before use.

The staining fluid is made up, according to Gram's original directions, as follows:

Five c.c. of anilin oil are shaken up thoroughly with 125 c.c. of distilled water. This solution is then filtered through a moist filter paper.

To 108 c.c. of this anilin water add 12 c.c. of a saturated alcoholic solution of gentian violet. The stain acts best when twelve to twenty-four hours old, but may be used at once. It lasts, if well stoppered, for three to five days. A more convenient and simple method of making up the stain is as follows:

To 10 c.c. of distilled water in a test-tube add anilin oil until on shaking the emulsion is opaque—roughly, 1 to 10. Filter this through a wet paper until the filtrate is clear. To this add saturated alcoholic solution of gentian violet until the mixture is no longer transparent and a metallic film on the surface indicates saturation. One part of alcoholic saturated gentian violet to nine parts of the anilin water will give this result. This mixture may be used immediately and lasts two to five days if kept in a stoppered bottle.

Cover the preparation with this; leave on for five minutes. Pour off excess stain and cover with Gram's iodine solution for two or three minutes.

GRAM'S IODIN SOLUTION.

Iodin	1.0 gram
Potassium iodide	2.0 grams
Distilled water	300.0 c.c.

Decolorize with 97 per cent. alcohol until no further traces of the stain can be washed out of the preparation. This takes usually thirty seconds to two minutes, according to thinness of preparation. Wash in water.

Counterstain with an aqueous contrast stain, preferably Bismarck brown or safranin.

Sterling's Modification of Gram's Method.—Two c.c. anilin oil and 10 c.c. 95 per cent. alcohol. Shake and add 88 c.c. distilled water. Five grams of gentian violet are ground in a mortar and the anilin solution added slowly while grinding. Filter. This solution keeps and stains in one-half to one minute.

CLASSIFICATION OF THE MOST IMPORTANT PATHOGENIC BACTERIA ACCORDING TO GRAM'S STAIN.

Gram-positive.

(Retain the gentian violet.)

Micrococcus pyogenes aureus
 Micrococcus pyogenes albus
 Streptococcus pyogenes
 Micrococcus tetragenus
 Pneumococcus
 Bacillus subtilis
 Bacillus anthracis
 Bacillus diphtheriæ
 Bacillus tetanus
 Bacillus tuberculosis and other
 acid-fast bacilli
 Bacillus aërogenes capsulatus
 Bacillus botulinus

Gram-negative.

(Take counterstain.)

Meningococcus
 Gonococcus
 Micrococcus catarrhalis
 Bacillus coli
 Bacillus dysenteriæ
 Bacillus typhosus
 Bacillus paratyphosus
 Bacillus fecalis alkaligenes
 Bacillus enteritidis
 Bacillus proteus
 Bacillus mallei
 Bacillus pyocyaneus
 Bacillus influenzæ
 Bacillus mucosus capsulatus
 Bacillus pestis
 Bacillus maligni edematis
 Spirillum cholerae
 Bacillus Koch-Weeks
 Bacillus Morax-Axenfeld

CARBOL-GENTIAN VIOLET.

Saturated alcoholic solution of gentian

violet	90.0 c.c.
Five per cent. phenol in water . .	1000.0 c.c.

This solution retains its staining powers for the Gram method of staining for a longer period than does the ordinary Gram solution, but is not as permanent as the Sterling modification.

POLYCHROME STAINS.—The Romanowsky stain depends on the formation of methylene azure and methylene violet in alkaline solution of methylene blue. When this solution is mixed with a solution of water-soluble yellowish eosin, the eosinates of methylene azure, methylene violet, and methylene blue are thrown down, as these eosinates are insoluble in water.

Wright's stain consists of a solution of these eosinates in methyl alcohol.

Any methylene blue and any yellowish water-soluble eosin issued by the Medical Department can be used in preparing the stain.

WRIGHT'S STAIN.—Add 1 gram of methylene blue to 100 c.c. of a 0.5 per cent. solution of sodium bicarbonate in water and heat for one hour, after steam is up, in an Arnold sterilizer. The flask containing the alkaline methylene blue solution should be of such size that the depth of the fluid does not exceed two and a half inches. When cool add to the methylene blue solution 500 c.c. of a 1 to 1000 eosin solution (yellowish eosin, water soluble). Add the eosin solution slowly, stirring constantly, until the blue color is lost and the mixture becomes purple, with a yellow metallic luster on the surface and there is formed a finely granular black precipitate. The precipitate is the water insoluble eosinates of methylene blue and of methylene azure and other oxidation products

of methylene blue. The end-reaction is reached when enough eosin has been added to neutralize all of the methylene blue and its oxidation products. To determine this end-reaction, place a drop of the mixture on a piece of filter paper, a slight eosin halo appears around the drop, due to a slight excess of eosin. As the precipitate is soluble in eosin, add only enough excess of eosin to get the slight halo. Collect this precipitate on a filter paper and dry in the incubator at 38°C . When thoroughly dry, dissolve 0.06 gram in 20 c.c. of pure methyl alcohol (acetone-free). This is the stock solution. For use, filter the 20 c.c. and add to the filtrate 5 c.c. of methyl alcohol.

The dry powder keeps well: the alcoholic solution does not keep well. Therefore it is better to make only enough of the solution to last a couple of months.

Method of Staining.—1. Make films and dry in the air. The film must dry quickly and must be protected from dust and dirt.

2. Cover the dry film preparation with the stain for one minute (to fix).

3. Add distilled water to the stain on the preparation, drop by drop, until a yellow metallic scum begins to form (to stain). Add the drops of water rapidly in order to prevent precipitates on the stained film; practically add 1 drop of water for every drop of stain used. Allow to stain for five to ten minutes.

4. Wash off the stain with distilled water.

5. Wash in distilled water until the film has a pinkish tint.

6. Blot dry with filter paper. Do not put on a cover-glass or mount in liquid petroleum.

Red cells are orange to pink; nuclei, various shades of violet; eosinophile granules are red; neutrophile granules are yellow to lilac; blood plates are purplish; malaria parasites: cytoplasm is blue and chromatin is metallic red to rose pink.

Caution.—Never heat a preparation that is to be stained

by Romanowsky, and use only distilled water or rain water, in all Romanowsky methods. Old distilled water should be boiled to drive off CO_2 , especially for Giemsa stain.

FONTANA METHOD FOR TREPONEMATA.

The following three solutions are used:

I

Acetic acid	1.0
Formalin	20.0
Distilled water	100.0

II.

Phenol	1.0
Acid tannic	5.0

III.

Twenty-five per cent. solution of silver nitrate	5.0 c.c.
Ammonia water	1.0 drop

Dry slide in air.

Wash in I for one minute.

Wash in water.

Pour on II and steam one-half minute.

Wash in water.

Pour on III, steam for one-half minute.

Wash in water.

Mount in balsam.

Immersion oil takes out the color.

CLEANSING SOLUTION FOR GLASSWARE.

Potassium bichromate (powdered)	200 grams
Water distilled, up to	1500 c.c.
Sulphuric acid conc.	500 c.c.

This solution may be used for cleaning test-tubes and other glassware used in the preparation of the Colloidal Gold

Reagent and the Lange test. Microscopic slides cleaned with this solution may be used repeatedly.

Method for preparation of chemically clean glassware:

1. Boil for thirty minutes in hot Ivory soap solution.
2. Brush thoroughly under hot tap water.
3. Rinse in running water for ten minutes.
4. Immerse in hot bichromate solution for thirty minutes.
5. Rinse in running water for ten minutes.
6. Rinse in ordinary distilled water.
7. Rinse with triply distilled water.

KLOTZ'S FLUID.

The chief advantage of Klotz's method of preserving pathological specimens is that the specimens may be preserved and shipped in Fluid No. 1.

A practical method for a Base Hospital with a large pathological service is to make up a liberal supply of Carlsbad salts with which the fluid is readily prepared.

Modified Carlsbad salts:

Potassium sulphate	40 grams
Sodium or potassium nitrate	760 grams
Sodium chloride	360 grams
Sodium bicarbonate	400 grams
Sodium sulphate	440 grams

Fix tissues three to five days or longer in Fluid No. 1:

Carlsbad salts	375 grams
Chloral hydrate	375 grams
Formalin	375 c.c.
Water	15 liters

Wash three to five hours in running water. Preserve in Fluid No. 2:

Carlsbad salts	375 grams
Chloral hydrate	150 grams
Formalin	75 c.c.
Water	15 liters

KAISERLING'S FLUID.

For preservation of colors of gross pathological specimens the following method gives satisfactory results:

1. Fix the tissue in following solution for one to five days in Kaiserling's Fluid No. 1:

Formaldehyde	200 c.c.
Water	1000 c.c.
Potassium nitrate	15 grams
Potassium acetate	30 grams

2. Drain and place in 80 per cent. alcohol for one to six hours.

3. Ninety-five per cent. alcohol for one to two hours.

4. Preserve specimen in Kaiserling's Fluid No. 3:

Potassium acetate	200 grams
Glycerine	400 c.c.
Water	2000 c.c.

NEUTRAL SODIUM HYPOCHLORITE SOLUTION
("DAKIN'S SOLUTION").

PREPARATION FROM BLEACHING POWDER.—*Dakin's Original Method.*—A strong solution of hypochlorite is prepared by decomposing 150 grams bleaching powder (about 25 to 35 per cent. available chlorine) with 105 grams dry sodium carbonate (122 grams monohydrate ($\text{Na}_2\text{CO}_3 \cdot \text{H}_2\text{O}$) or 284 grams washing soda ($\text{Na}_2\text{CO}_3 \cdot 10\text{H}_2\text{O}$)). The mixture is very thoroughly shaken, both to make good contact and to render

the precipitated calcium carbonate granular and promote its settling. It is then allowed to stand quietly and after half an hour the clear liquid is siphoned off from the precipitate and filtered through paper or a cotton plug.

A 10 c.c. portion is rapidly titrated with $\frac{N}{2}$ boric acid solution (31 grams per liter), using powdered phenolphthalein as indicator (the usual alcoholic solution of phenolphthalein will not serve) in order to determine the amount of boric acid to be added to the rest of the filtrate. The end-point is the disappearance of the pink color. Each cubic centimeter of $\frac{N}{2}$ boric acid required for the 10 c.c. sample calls for the addition of 3 grams boric acid per liter of filtrate. An excess of boric acid should be avoided, as it favors the liberation of hypochlorous acid and renders the solution less stable. It is best to add slightly less than the calculated amount. The concentrated solution thus prepared contains about 4 per cent. of sodium hypochlorite, and before use should be diluted with about 7 parts of water and titrated with $\frac{N}{10}$ thiosulphate to determine its precise hypochlorite concentration. It is then accurately diluted to the required strength (usually 0.5 to 0.45 per cent.).

PREPARATION FROM CHLORINE AND SODIUM CARBONATE.—Chlorine may be obtained as the compressed gas in steel cylinders and is easily measured by a chlorine meter manufactured for the purpose. This is a stable, economical and convenient source of chlorine. A solution is prepared containing 15 grams of dry sodium carbonate per liter (= 17.6 grams monohydrate or 40 grams of washing soda.) A measured quantity, 4.8 grams (or about 1600 c.c.) of chlorine gas is allowed to run into the solution for each liter. Ten c.c. of the solution is then titrated. If the solution is too weak more chlorine is introduced. If the solution is too strong it should be diluted to a concentration of 0.5 per cent. NaOCl with 1.5 per cent. sodium carbonate solution, which at the same time serves to correct the unduly diminished alkalinity

caused by the excess of chlorine introduced into the solution. If the final solution fails to give a momentary flash of color with alcoholic solution of phenolphthalein it should be rejected. If the solution shows color with powdered phenolphthalein it must be titrated with boric acid as described above, for preparation from Bleaching Powder, until this defect is corrected, or it must be discarded. The solution should be titrated for hypochlorite concentration every twenty-four to forty-eight hours and discarded when it falls below the desired lower limit (usually 0.45 per cent.).

If a chlorine meter is not available, chlorine gas may be run into the 1.5 per cent. carbonate solution through any improvised diffuser until the hypochlorite concentration has reached 0.5 per cent. The amount of chlorine required to give a hypochlorite concentration of 0.5 per cent. is approximately twice the amount required to cause decolorization of powdered phenolphthalein. It is therefore convenient to add powdered phenolphthalein and note the amount of chlorine required to cause its decolorization. When almost twice that amount of chlorine has been introduced, frequent titrations of the hypochlorite content must be commenced to determine the proper point at which to stop the introduction of the chlorine.

TITRATION OF DAKIN'S SOLUTION.—To 10 c.c. of the Dakin solution, add approximately 5 c.c. of a 10 per cent. solution of potassium iodid and 3 c.c. of glacial acetic acid. Iodin is liberated and dissolves in the excess of iodid present. Dilute with water to about 50 c.c. A standard $\frac{N}{10}$ thiosulphate solution is then added from a burette until the solution is just rendered colorless. The number of cubic centimeters required to effect this result multiplied by the factor 0.0372 gives the percentage of sodium hypochlorite present in the Dakin solution.

PREPARATION OF STANDARD $\frac{N}{10}$ THIOSULPHATE SOLUTION.—Dissolve exactly 24.82 grams of pure crystalline sodium thiosulphate in water and make up to exactly 1 liter. One c.c. of

this standard solution is equivalent to 0.00372 gram of sodium hypochlorite.

TITRATION OF BLEACHING POWDER.—Bleaching powders vary considerably in their "available chlorine" content, so that it is desirable to determine the available chlorine in each large batch. Bleaching powders with less than 20 per cent. available chlorine should be rejected. Exceptional samples may contain as high as 35 per cent. available chlorine.

The available chlorine content may be determined as follows: Exactly 10 grams of bleaching powder made up of small samples from different parts of the jar, in order to obtain a representative sample, are well shaken with a liter of water. After standing about six hours the solution is filtered and a 10 c.c. sample of the filtrate is titrated in exactly the same manner as in the titration of Dakin's solution. In this case the number of cubic centimeters of decinormal thiosulphate required to decolorize, multiplied by the factor 3.55, gives the percentage of active chlorine in the bleaching powder.

OTHER CHLORINE ANTISEPTICS.

Antiseptics of the chlorine group when properly applied have proved to be the most efficient antiseptics used in the present war. We are indebted to Dakin for two other important chlorine antiseptics:

"CHLORAMIN-T."—Chloramin-T is the abbreviated name for sodium-toluene-sulphon-chloramin. Chloramin-T is soluble in water and can be used in stronger solution (up to 2 per cent.) than the hypochlorites. It is more stable and exerts more prolonged action than hypochlorite when acting in the presence of much blood. It is not toxic and is less irritating than the hypochlorites, but it has but little solvent action on necrosed tissue. It is well suited for use on wounds previously cleansed with hypochlorites, and in suitably dilute

¹ Dakin and Dunham: Hand-book of Antiseptics.